

The Incidence of Methicillin Resistance *Staphylococcus aureus* in Diabetic Septic Foot

Mahjoob Osman Mahjoob*, Abualgasim Elgaili Abdalla, Haitham E Elawad, Alla Alamin Abdulla, Saffa Omer Altayeb, Umalhassan Hashim Abdalla

Department of Microbiology, Medical Laboratories Sciences, Omdurman Islamic University, Omdurman, Sudan

ABSTRACT

Background: Foot infection is the most common problem in a person with diabetes. Infection in these patients is difficult to treat because these individuals have impaired microvascular supply, Which limits the access of phagocytic cell in the infected area and result in poor concentration of antibody in the infected tissue. *Staphylococcus aureus* is a major cause of potentially life-threatening infection which was acquired in the health care and community setting. These microorganisms have developed resistance against most classes of antibacterial agent as indicated by a dramatic increase in the number of healthcare-associated infection due to methicillin resistance *S. aureus*, which become endemic in hospitals worldwide.

Methods: All collected samples were cultured directly on the blood and chocolate agar to primary isolation, then purified by several subcultures from a single well-isolated colony. Identification of isolated bacteria depends on gram reactions, organism morphology, colonial morphology in different media and biochemical tests-catalase test, coagulase test, DNase test, mannitol fermentation test, and VP test. The antimicrobial test was carried by disc diffusion method Kirby-Bauer method on Muller and Hinton media to several single antibiotic discs-Vancomycin, cloxacillin, Tobracillin, Ciprofloxacin, and Ceftriaxone. The inhibition zone was measured by ruler in millimeter the compared with incorporated chart.

Results: A total of 50 diabetic patients with wound infection were including *S. aureus* n:20, 40% and other pathogens n:30,60%. Frequency of male was 43,86% while 7,14% were female. All patient were classified into two groups of age, one (35-55) with moderate frequency n:20, 40% and another age group (56-90) with the highest frequency n:30,60%. All isolated *S. aureus* were resisted cloxacillin while sensitive to vancomycin, 60% of them resist tobracillin 40% were sensitive and 10% resist ciprofloxacin 90% were sensitive, 25% were resisted ceftriaxone 75% were sensitive.

Conclusion: MRSA is highly 40% prevalent among populations of *S. aureus* isolated from surgical site infection in Khartoum state. This study discusses the link between MRSA acquisition factors like age, sex, occupation, ethnicity, geographical location, hospitalization, antibiotic use, surgery and distinction community-acquired MRSA and hospital-acquired MRSA.

Keywords: Diabetic; *S. aureus*; Septic foot

Abbreviation: MRSA: Methicillin Resistance *Staphylococcus aureus*

Correspondence to: Mahjoob Osman Mahjoob, Department of Microbiology, Medical Laboratories Sciences, Omdurman Islamic University, Omdurman, Sudan, Tel: +249914449443; E-mail: Mahjoob212@hotmail.com

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INTRODUCTION

Infection is the most common problem in a person with diabetes. These individuals are predisposed to foot infection because of a compromised vascular supply secondary to diabetic. local trauma and/or pressure (Often in association with lack of sensation because of neuropathy), in addition to microvascular disease, may result in various diabetic foot infections that run in the spectrum from simple, superficial and cellulitis to chronic osteomyelitis [1]. Infection in patients of diabetes is difficult to treat because these individuals have impaired microvascular circulation which limits the access of phagocytic cell to the infected area and result in the poor concentration of antibiotics in infected tissue [1-4]. The wound in diabetics characterized by pus, purulent discharge, foul smell, yellowish and then complicated may lead to amputation. *S. aureus* is a major cause of potential life-threatening infection which was acquired in health care and community setting. The organism has developed resistance against most classes of antimicrobial agents as indicated by dramatic increase in the number of healthcare-associated infections due to methicillin resistance *S. aureus* [5]. Over the last decade MRSA strains have become endemic in hospitals worldwide. In addition, it is now incipient community pathogen in many geographical regions [6]. Glycopeptides such as vancomycin (derived from *Streptomyces Orientalis*) are frequently the antibiotics of choice for treatment of infection caused by MRSA [7].

OBJECTIVES

To show the incidence of MRSA in diabetic septic foot.

MATERIALS AND METHODS

Enrolment of patients

Written consent of this study was obtained from every patient before being enrolled in the study.

Sample collection

50 wound cotton swabs deepened in Stuart's transport media.

Phenotypic characterization of the isolates

Culture media: All collected samples were cultured directly in blood agar and chocolate agar to primary isolation then the inoculated plate was incubated for 24 hours at 37°C [8]. Then sub-cultured in Mannitol Salt Agar and incubated at 37°C after overnight incubation yellow colonies indicated mannitol fermentation.

Purification: All isolated bacteria were purified by several subculturing to single well isolating colony. The purity of isolates was checked by examining the gram stain.

Identification of bacteria

Gram staining technique: The pure colonies were emulsified in the drop of D.W in clean dry microscopically slide, and allowed

to dry. Then dried smear was fixed by passing three times over the flame.

Staining procedure: The fixed smear was covered with crystal violet solution for one minute, then was washed with tap water and covered with gram iodine solution for one minute. The smear was washed with acetone alcohol solution for few seconds, then washed with tap water. The smear was covered with safranin solution for two minutes, then washed with tap water, and allowed to dry. The smear was examined with the oil immersion lens [8].

Biochemical test

Catalase test: It was carried out by tube method, human plasma was diluted as 1 in 10 normal salines, then poured into a sterile test tube in 0.5 ml amount, pure colonies were transferred into plasma tube and incubated at 37°C up to 6 hours. The positive test indicated by clotting of the plasma [9].

DNase test: The tested organism was potted on to DNA medium and incubated at 37°C, after overnight incubation, the culture plate was flooded by 1 mol/l Hydrochloric Acid (HCL) solution which precipitating unhydrolyzed DNA, the action of DNase enzyme was observed by clearing around the colonies [9].

VP test: The test organism was inoculated into glucose phosphate peptone water and incubated at 37°C after overnight incubation alpha-naphthalamine and potassium hydroxide were added, and the tube was exposed to air. A positive reaction was indicated by developing red color complex [9].

Antimicrobial susceptibility: It was carried out by disc diffusion (Karry-Blair), Normal saline suspension of tested organism was inoculated on Muller-Hinton Agar by swabbing all agar surface with cotton swab, single several discs (vancomycin 30 mg, cloxacillin 5 mg) were then placed on agar surface with sterile forceps, after 24 hours incubation at 37°C the inhibition zone was measured by ruler in millimetre, then compared with incorporated chart [9].

RESULTS

Isolation of organisms

A total of 50 diabetics patients with symptoms of wound infection were including *S. aureus* (n:20, 40%) and other pathogens (n:30, 60%) (Table 1).

Table 1: Distribution of isolated organism among diabetic's patients with symptoms of wound infection.

Isolated organism	No.	%
<i>S. aureus</i>	20	40%
Other pathogens	30	60%
Total	50	100%

Gender

Among the study population (n:50) who were suspected to have an infection with MRSA, the frequency of male was 43 (86%) while 7 (14%) were females.

Age group

All patient were classified into 2 age group, age group one (35-55) with moderate frequency (n:20, 40%) and age group two (56-90) with the highest frequency (30, 60%).

The results of antibiotics susceptibility test of *S. aureus*

Cloxacillin 100% were resistance, Vancomycin 100% were sensitive, Tobracillin 40% were sensitive while 60% were resistance, Ciprofloxacin 90 were sensitive while 10% were resistance and Ceftriaxone 75% were sensitive and 25% were resistance (Figure 1).

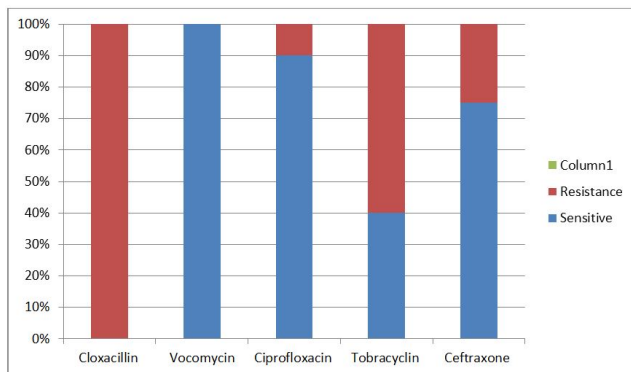


Figure 1: Antibiotics susceptibility result of different antibiotics against *S. aureus*.

DISCUSSION

Community-acquired MRSA is a rapidly emerging public health concern. The prevalence of community-acquired MRSA in the general population is known to be increasing and extending worldwide. It is probably underreported because submission of the skin and soft tissue specimens for culture has not been routine clinical practice. Our study showed a similar result with 40% but a higher prevalence of MRSA in the ICU at approximately 80%. Our results could mean that the prevalence of MRSA in our institution was lower compared to the prevalence rate in the USA or that the true prevalence rate is not reflected due to the small number of isolate included in the study. Our study in the other hand showed a high prevalence rate 40% that we disagree with the percent of MRSA published in the 1999 antimicrobial resistance surveillance data 17.4%. The 40% *Staphylococcus aureus* reported in the present study may depict the level of *Staphylococcus aureus* carriage in this locality;

be attributed to the level of contamination arising from the habit of some of the volunteer to treat their wound aseptically before seeking appropriate medical attention and may also be due to low personal hygiene and poor health education which still persist in Sudan compared to other countries. Regarding the gender distribution in our study and out of 50 isolates, 43 (86) male patients were infected more than 7 (14%) female, agree with male 60% and female 40%, male 63% and female 37%. This confirms earlier report that most tropical bacterial infection is more common in male than female because predisposing site for the infection such as overpopulation, prisons, military camps, construction site, and factories are predominant by males.

Regarding the distribution of age of patients (56-90) 60% agree with Stephen Stanway 61%. Among 20 *Staphylococcus aureus* 20 were MRSA by routine disc diffusion test and all these 20 were sensitive to vancomycin these values agree with 92.2%.

CONCLUSION

MRSA is highly (40%) prevalent among the population of *S. aureus* isolated from surgical site infection in Khartoum state. This study discusses the link between MRSA acquisition and various factors like age, sex, antibiotic usage, surgery and distinction between community-acquired MRSA and hospital-acquired MRSA.

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